

Clean Copy of Amended Claims

A¹
1 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the *Pinus* subgenus with *Agrobacterium* for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

2 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;

(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

3 (amended). The method of claim 2, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

4 (amended). The method of claim 1, wherein said damage to cells is minimized by:

(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;

(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

A1
could
5 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

6 (amended). The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

A2
9 (amended). The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

A3
12 (amended). The method of claim 1, wherein said selection is performed by culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

A4
15 (amended). The method of claim 14, wherein said layer is a layer of liquid medium.

16 (amended). The method of claim 14, wherein said layer is a layer of gelled medium.

A5
19 (amended). The method of claim 1 which further comprises the eradication of *Agrobacterium* from the pine cells after incubation with *Agrobacterium*.

20 (amended). The method of claim 19, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

A5
amended

21 (amended). The method of claim 20, wherein said layer is a layer of liquid medium.

22 (amended). The method of claim 20, wherein said layer is a layer of gelled medium.

A4

25 (amended). A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the subgenus *Pinus* with *Agrobacterium* for *Agrobacterium* transformation;

eradicating *Agrobacterium* from the pine cells after incubation with *Agrobacterium*;

minimizing damage to cells subsequent to *Agrobacterium* infection, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

26 (amended). The method of claim 25, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a liquid wash medium;

(b) agitating said liquid wash medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

27 (amended). The method of claim 26, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

28 (amended). The method of claim 26, wherein said selection is performed by

culturing cells which have been incubated with *Agrobacterium* on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and
selecting transformed cells.

29 (amended). The method of claim 26, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

30 (amended). The method of claim 28, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

31 (amended). The method of claim 25, wherein said damage to cells is minimized by:
(a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
(b) rinsing said cells using a liquid wash medium to remove *Agrobacterium*; and
(c) recovering washed cells with minimal damage.

32 (amended). The method of claim 31, wherein pine cells are plated onto a support
membrane prior to *Agrobacterium* transformation.

33 (amended). The method of claim 31, wherein pine cells are plated onto a support
membrane subsequent to *Agrobacterium* transformation.

34 (amended). The method of claim 31, wherein said selection is performed by
culturing cells which have been incubated with *Agrobacterium* on a support membrane
placed over a gel medium;

Ag
cont'd

contacting said cells with a selection agent; and
selecting transformed cells.

35 (amended). The method of claim 31, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

36 (amended). The method of claim 34, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

37 (amended). The method of claim 25, wherein said selection is performed by
culturing cells which have been incubated with *Agrobacterium* on a support membrane
placed over a gel medium;
contacting said cells with a selection agent; and
selecting transformed cells.

38 (amended). The method of claim 25, wherein said eradication is performed by:
culturing cells which have been incubated with *Agrobacterium* on a support membrane over
a layer containing an eradicator, said layer in or positioned over a gel medium; and
recovering cells from which said *Agrobacterium* has been eradicated.

39 (amended). A method for minimizing damage to transformed cells of pine of the genus
Pinus subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation
which comprises:

(a) washing transformed cells of the subgenus *Pinus* in a liquid wash medium;

AP
critical

- A6
cancel
- (b) plating said cells on a support membrane;
 - (c) suspending said cells in a liquid wash medium; and
 - (d) recovering washed cells with minimal physical damage.
-

A7

43 (amended). The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the wash medium, said wash carried out for between half an hour to overnight in duration.

A8

46 (amended). A method for pine cell tissue culture which comprises culturing pine cells of the genus *Pinus* subgenus *Pinus* on a support membrane placed over a gel medium.

47 (amended). The method of claim 46, wherein said support membrane is placed over a layer containing one or more tissue culture medium constituents, said layer is positioned on said gel medium.

A9

49 (amended). The method of claim 47, wherein said layer is a layer of liquid medium.

A10

52 (amended). A method for selecting transformed cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

- culturing cells of the *Pinus* subgenus subsequent to transformation on a support membrane placed over a gel medium;
- contacting said cells with a selection agent; and
- selecting transformed cells.

A11

55 (amended). The method of claim 54, wherein said layer is a layer of liquid medium.

A12

58 (amended). A method for eradicating *Agrobacterium* from cells of pine of the genus *Pinus* subgenus *Pinus* which comprises:

culturing cells of the *Pinus* subgenus on a support membrane over a layer containing an
eradicant, said layer positioned in or over a gel medium; and
recovering cells from which said *Agrobacterium* contaminant has been eradicated.

59 (amended). The method of claim 58, wherein said layer is a layer of liquid medium.

60 (amended). The method of claim 58, wherein said layer is a layer of gelled medium.

A12
cond 2